PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BW274R/RCGE/rmp	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IT 03/00132	International filing date *(day/month/year)* 05.03.2003	Priority date *(day/month/year)* 05.03.2002
International Patent Classification (IPC) or both national classification and IPC A61K38/18		
Applicant GEYMONAT S.p.A et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.10.2003	05.04.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Böhmerova, E Telephone No. +49 89 2399-7859

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IT 03/00132

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-15 as originally filed

Claims, Numbers

1-15 received on 13.02.2004 with letter of 11.02.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-15
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Cited documents

Reference is made to the following documents:

D1: Maglione et al., Il Farmaco, Societa Chimica Italiana, Pavia, It (2000), 55, 165-167

D2: Ziche et al., Laboratory Investigation, United States And Canadian Academy Of Pathology, Baltimore, US, (04-1997), 76(4), 517-531

Unless indicated otherwise reference is made to the passages considered relevant in the search report.

Novelty

Claims 1-10

The subject-matter of claims 1-6 is novel under Article 33(1) and (2) as none of the cited documents discloses the use of PLGF-1 for the treatment of diseases or pathological alterations involving the cutaneous or subcutaneous connective tissue, scleroderma, early skin ageing and hair loss.

D1 shows that PLGF-1 has angiogenic activity and provides a protective effect against myocardial lesions induced by isoprenalin. As the use of PLGF-1 for the preparation of medicament for preventive or curative treatment of diseases including "pathological alterations involving internal organ connective tissue and/or the vascular system" was excluded from the scope of the claims, the disclosure of D1 is no longer prejudicial for novelty of claims 1-6.

The subject-matter of claims 7-10 is novel under Article 33(1) and (2) as none of the cited documents discloses a cosmetic use of PLGF-1.

Claims 11-13,15

The subject-matter of claims 11 - 15 is considered as being novel under Article 33(1) and (2) for the following reasons:

D1 does not disclose compositions comprising at least 98.5% of PLGF-1 in the active dineric and multimeric form.

D2 discloses a corneal implant or a solution comprising purified PLGF-1. The purified PLGF-1 is in the form of a solution comprising NaCl and Tween or is lyophilised and it comprises 0.4% of monomeric, 86,3% of dimeric and 13,2% of trimeric form. The corneal implants used in D2 comprise maximum 200 ng or PLGF-1 per pellet representing a dosage unit. The scope of independent claim 11 directed to a pharmaceutical composition is restricted to the pharmaceutical compositions comprising PLGF-1 in an amount from 50 µg to 30 mg per unitary dose for parenteral use which is an amount higher than the amounts defined in D2. The solution of D2 comprises maximum 100 ng of PLGF-1 per ml. The scope of claim 12 is restricted to the cosmetic composition comprising PLGF-1 in an amount from 0.01 mg to 0.09 mg per gram of composition which are again above the amount disclosed in D2. Consequently, the subject-matter of claim 11 as well as claims 13 - 15 dependent thereon is novel over the disclosure of D2.

Inventiveness

The subject-matter of claims 1 - 10 is considered to be inventive under Article 33(1) and (3) for the following reasons:

The problems to be solved by the present application can be defined as to provide:
- a medicament for treating diseases involving cutaneous or subcutaneous connective tissue, scleroderma or early skin ageing
- a medicament for the preventive or curative treatment of pathological loss of hair
- a cosmetic agent for prevention and cosmetic treatment of natural skin ageing
- a cosmetic agent for the cosmetic treatment of the natural loss of hair.

The solution proposed by claims 1-10 is the use of PLGF-1.

The experimental data present in the application are considered to be sufficient to prove that the claimed solution actually solves the above defined technical problems, see Example 6 for scleroderma and Example for skin ageing. Example 7 shows that the capillary vascularization is increased particularly in the hair perifollicular areas. The description (page 8, paragraph 4) further teaches that an increase in the hair bulb dimensions and in the hair diameter itself was observed after the local application of PLGF-1. It is known in the art that the increase in the vascularisation of the hair follicle stimulates its growth (see page 15, line 24-26) and the angiogenic activity of PLGF-1 is known from D2. However, taking into the consideration the known "general" angiogenic activity of PLGF-1, it was not possible to predict that PLGF-1 would selectively increase the vascularisation of the hair follicle or that it would increase the hair bulb dimension and hair diameter.

It is considered that there is no disclosure in the prior art leading a skilled person to the use of PLGF-1 in the treatment or prevention of the disease states as claimed in claims 1-6 or to the cosmetic use as claimed in claims 7-10.

The technical problem underlying claims 11-15 in the light of D2 is to provide means for treating or alleviating conditions involving alteration of the connective tissue. The solution proposed by the application is the use of PLGF-1 compositions with the level of dimerisation/multimerisation and unite dosage/concentration as claimed in claims 11 and 12. As already stated above, the claimed compositions differs over those disclosed in D2 in that they comprise higher amounts of PLGF-1 per dosage unit or per gram of composition. Example 6 shows that in order to achieve a recognizable effect, high amounts of medicament have to be given. No effect was achieved by daily injection of PLGF-1 at 0.1 µg/ml. Consequently, the identification of the effective amounts PLGF-1 for the treatment of alterations of connective tissues can be acknowledged as the feature conferring inventiveness to the subject-matter of claims 11-15.

Industrial applicability

The subject-matter of independent claims 1-15 is considered to be industrially applicable under Art. 33(1) and (4) PCT.

CLAIMS

1. Use of type 1 Placental Growth Factor (PLGF-1) for the preparation of a medicament promoting angiogenesis in the preventive or curative treatment of:

- diseases or pathological alterations involving the cutaneous or subcutaneous connective tissue, or
- scleroderma, or
- early skin ageing due to exposure to atmospheric aggressive agents or to protracted solar irradiation.

2. Use of type 1 Placental Growth Factor (PLGF-1) according to claim 1, wherein the disease is localised scleroderma or progressive systemic scleroderma.

3. Use of type 1 Placental Growth Factor (PLGF-1) according to claim 2, wherein the localised scleroderma is cutaneous and the progressive systemic scleroderma is myocardial scleroderma.

4. Use of type 1 Placental Growth Factor (PLGF-1) according to claim 1, for the preparation of a medicament promoting angiogenesis in the preventive or curative treatment of the pathological loss of hair due to alopecia, hormonal disorders, chemotherapy, radiotherapy or medicament administration.

5. Use according to any one of claims 1 to 4, wherein the medicament is in a form suitable for generating a local or systemic effect.

6. Use according to any one of claims 1 to 5, wherein the medicament is in the form suitable for endovenous, intramuscolar, intrarticular, subcutaneous administration, topical administration or by subcutaneous implant or ionophoresis.

7. Use of type 1 Placental Growth Factor (PLGF-1) as promoter of cutaneous or subcutaneous angiogenesis in the prevention and cosmetic treatment of natural skin ageing.

8. Use of type 1 Placental Growth Factor (PLGF-1) as promoter of perifollicolar angiogenesis in the prevention and in the cosmetic treatment of the natural loss of hair.

9. Use according to any one of claims 7 or 8, wherein the PLGF-1 is formulated in a cosmetic composition for topical administration.

10. Use according to any one of claims 1 to 9, wherein PLGF-1 is comprised in an amount suitable for an administration of 1 to 500 µg per Kg of body per day, preferably of 10 µg/Kg/day to 200 µg/Kg/day.

11. Pharmaceutical composition comprising PLGF-1 as active principle and a pharmaceutically acceptable excipient, characterised in that at least the 98.5% of the PLGF-1 is in active dimeric and multimeric form, at least the 70% is in dimeric form and no more of the 1.5% is in monomeric form, and in that PLGF-1 is comprised in an amount from 50 µg to 30 mg per unitary dose for parenteral use and in an amount from 0.1 mg to 10 mg per gram of composition for topical use.

12. Cosmetic composition comprising PLGF-1 as active principle and a cosmetically acceptable excipient, characterised in that at least the 98.5% of the PLGF-1 is in active dimeric and multimeric form, at least the 70% is in dimeric form and no more of the 1.5% is in monomeric form, and in that PLGF-1 is comprised in an amount from 0.01 mg to 0.09 mg per gram of composition.

13. Composition according to claims 11 or 12, characterised in that PLGF-1 is an expression product from genetically modified host cells (page 5, lines 23, 24) obtained in accordance with the method disclosed in the application PCT/IT02/00065 (WO-A-03/066676).

14. Pharmaceutical or cosmetic composition according to claim 11 to 13, characterised in that it is for local or systemic use and is in form of solution, lotion, W/O emulsion, O/W emulsion, suspension, liposome suspension, gel, cream, paste, ointment or subcutaneous implant.

15. Composition according to any one of claims 11 to 14, comprising one or more substances capable of stabilising the PLGF-1 in the active dimeric-multimeric forms.